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Recombinant Gamma Interferon Induces HLA-DR Expression on Cultured Human Keratinocytes*

TERESA Y. BASHAM, PH.D., BRIAN J. NICKOLOFF, M.D., PH.D., THOMAS C. MERIGAN, M.D., AND VERA B. MORHENN, M.D.

Division of Infectious Diseases, Department of Medicine (TYB, TCM) and Department of Dermatology (BJN, VBM), Stanford University School of Medicine, Stanford, California, U.S.A.

In normal human epidermis, expression of HLA-DR antigen is restricted to Langerhans cells (LC) and acrosyringial epithelium. However, in diseases such as lichen planus and graft-vs.-host, HLA-DR antigen appears to be expressed by keratinocytes, although the exact source of the HLA-DR is unclear. Two possibilities are that (1) the HLA-DR is shed by neighboring immunocompetent cells, or (2) that the keratinocytes are synthesizing the antigen themselves. Recently, gamma interferon has been shown to induce HLA-DR biosynthesis and expression on human malignant melanoma cells lines and on normal vascular endothelium. We report here that pure recombinant human gamma interferon (100 units/ml) induces HLA-DR expression on 60–70% of cultured human adult keratinocytes depleted of LC within 2–4 days of culture as determined by fluorescence-activated cell sorter (FACS) analysis using monoclonal antibodies. No residual LC or lymphocytes could be detected in these cultures. This is the first demonstration of HLA-DR expression by cultured human keratinocytes. This expression may be of functional significance in antigen presentation and cell-mediated cytotoxicity involving the epidermis.

Expression of the class II major histocompatibility complex antigen (HLA-DR) is required for antigen presentation [1] and induction of cytolytic T lymphocytes [2]. In normal human epidermis HLA-DR is expressed only on Langherhans cells (LC) [3] and acrosyringial epithelium [4]. The dendritic LC is thought to function as the primary antigen-presenting cell in the epidermis. However, keratinocytes express HLA-DR in a wide variety of diseases which include allergic contact dermatitis [5], lichen planus [6], and graft-vs.-host disease [7]. The dermis in these diseases contains a relatively dense, predominantly T-lymphocyte infiltrate. It is not known whether the HLA-DR is a product of these immunocompetent cells or the keratinocytes themselves.

Frozen sections from herpes labialis lesions demonstrate that within 2 days of recurrence, the entire epidermal layer stains positive for HLA-DR and infiltrating lymphoid cells are positive for gamma interferon (T. Cunningham, personal communication). Gamma interferon, which is produced by T lymphocytes [8] has recently been shown to induce HLA-DR biosynthesis and expression on human malignant melanoma lines [9] and cultured human vascular endothelium [10]. These findings suggested to us that gamma interferon, which has been shown to be a powerful immune regulator as well as an antiviral and antiproliferative agent, may be capable of inducing HLA-DR expression on normal adult keratinocytes. We report here that recombinant gamma interferon (r-IFN-γ) induces HLA-DR expression on cultured human adult keratinocytes depleted of LC. The HLA-DR expression by keratinocytes may functionally increase the antigen-presenting capacity of the epidermis in allergic contact dermatitis or signal keratinocyte destruction by T lymphocytes as seen in lichen planus and graft-vs.-host disease.

MATERIALS AND METHODS

Interferon

Human r-IFN-γ from *Escherichia coli* was generously supplied by Dr. G. Burton (Genentech, San Francisco, California). The titer of the r-IFN-γ as determined by virus inhibition plaque assay was 5×10^7 reference units/ml (U/ml) and the specific activity was 1×10^7 U/mg.

Preparation of Dispersed Skin Cells

Single cell suspensions of normal skin were prepared from facial skin obtained at surgery [11]. Trimmed skin was cut into 1 × 5 cm strips and split-cut with a Castroviejo keratotome set at 0.1 mm. The resulting slices were treated for 25 min at 37°C with 0.3% trypsin plus 0.1% EDTA in GNK (0.8% NaCl, 0.04% KCl, 0.1% glucose, 0.084% $NaHCO_3$, pH 7.3). Dispersed cells were suspended in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal calf serum (FCS), 50 μg/ml gentamicin, and 2 mM L-glutamine (complete medium). Viability, as determined by trypan blue exclusion immediately after trypsinization, was 90% or better. Small, round viable cells (2×10^6) were seeded on 3.5 cm collagen-coated Petri dishes (Lux, Flow Lab) in complete medium [11]. The cells were maintained in a humidified incubator with 5% CO_2/95% air at 37°C [11]. For all experiments, r-IFN-γ was added at 48 h after cell seeding. All time points indicated for harvesting and staining of cultures refer to this time point as day 0.

Panning

A 100 × 25 mm Lab Tek Petri dish (Scientific Products) was coated with goat antimouse immunoglobulin (Tago, Inc., Burlingame, California) at 10 μg/ml in 0.05 M Tris buffer, pH 9.5, incubated for 40 min at room temperature and then washed 3 times with Dulbecco's phosphate-buffered saline (DPBS) and once with 5% FCS/PBS [12]. The epidermal cells were incubated with antibody OKT6 (Ortho Diagnostic Systems) for 20 min, washed, resuspended in 5% FCS/PBS, layered on the coated Petri dish surface, incubated at 4°C for 40 min, swirled, and incubated for another 30 min. The supernatant containing unattached cells was poured off gently, and the Petri dish surface washed once with 1% FCS/PBS. The cells in the first wash were pooled with the supernatant cells and seeded exactly as described above.

Fluorescein Labeling and FACS Analysis

The cultured cells were washed to remove nonattached cells and the monolayer was trypsinized for 10 min using 0.3% trypsin plus 1%

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Reprint requests to: Vera B. Morhenn, M.D., Department of Dermatology, Stanford University School of Medicine, Stanford, California 94305.

Abbreviations:
DPBS: Dulbecco's phosphate-buffered saline
FACS: fluorescence-activated cell sorter
FCS: fetal calf serum
FITC: fluorescein isothiocyanate-conjugated
HLA: class II major histocompatibility complex antigen
LC: Langerhans cell(s)
r-IFN-γ: recombinant gamma interferon

EDTA and washed with DPBS containing Ca^{2+} and Mg^{2+}. Aliquots of 5×10^5 to 10^6 viable cells were indirectly stained for 20 min on ice with 1 μg of anti-HLA-DR monoclonal antibody L243 (IgG_{2a}) (Becton Dickinson Monoclonal Center, Inc., Mountain View, California) and 0.5 μg of OKT6 (IgG_1) diluted in heat-inactivated FCS containing 0.1% sodium azide. Nonspecific and Fc receptor-mediated background staining was determined either by omitting first-step antibody, L243 or OKT6, or substituting control isotypes for the first step. The cells were then washed twice with DPBS and stained for 20 min with 1 μg fluorescein isothiocyanate-conjugated (FITC) goat antimouse IgG monoclonal antibody (Becton-Dickinson, Mountain View, California). The labeled cells were diluted with DPBS and centrifuged through a layer of heat-inactivated FCS. The cells were then washed with DPBS and fixed with 1% paraformaldehyde in PBS. The fluorescence per cell was determined with a fluorescence-activated cell sorter (FACS III; Becton-Dickinson), and a histogram showing the number of stained cells against the intensity of fluorescence was recorded.

In situ immunofluorescence was accomplished following the above procedure, omitting the trypsinization. Coverslips were placed over the cells attached to the culture dishes and the cultures were photographed with a Leitz Dialux-20 microscope.

RESULTS

Primary cultures of keratinocytes from 3 different adult patients were treated with r-IFN-γ (100 U/ml) 48 h after seeding. The cultures were trypsinized and stained with a monoclonal antibody against HLA-DR. These cells were analyzed for both mean expression of HLA-DR per cell and the percent of positive cells per culture (Table I). R-IFN-γ enhanced the mean expression of HLA-DR (70–130) and increased the percent of cells stained to 50–70% by day 4 as seen in the histogram and the FACS analysis (Fig 1). We have found that this staining on day 4 represents near maximal HLA-DR expression by cultured keratinocytes.

To eliminate the possible shedding of HLA-DR from LC and subsequent adsorption by keratinocytes, cell suspensions were depleted of LC by panning prior to seeding. We have previously demonstrated that LC-depleted keratinocytes contain fewer than 1% LC [12]. On day 4, these cultures were stained and analyzed for OKT6 expression. No significant residual OKT6-positive cells could be detected at that time (Table I). Enhancement of HLA-DR expression was similar to that seen in the undepleted keratinocyte cultures (Table I).

Neither LC depletion nor r-IFN-γ resulted in gross morphologic changes in the attached keratinocyte monolayers (Fig 2*a*). These cultured cells are hexagonal with round nuclei and prominent nucleoli, typical of cultured keratinocytes. Since rounded-up clusters of keratinocytes occasionally remained attached to the underlying monolayer, we performed in situ staining. Fig 2*b* shows the immunofluorescence HLA-DR staining pattern observed on a day-4 LC-depleted culture with a phase microscopic view of the identical field (Fig 2*c*). This intense, rather uniform cell surface fluorescence of the hexagonal, attached cells characterized the majority of HLA-DR-positive keratinocytes. The r-IFN-γ-treated cultures stained in situ on day 4 with goat antimouse IgG-FITC alone as a control showed no specific fluorescence.

DISCUSSION

This is the first demonstration of HLA-DR expression by cultured human keratinocytes. Normal cultured epidermal cells do not synthesize HLA-DR after 7 days in culture, which presumably reflects the lack of growth of LC and acrosyringium in the culture dishes [13]. In order to eliminate the possible shedding of HLA-DR by LC and adsorption by keratinocytes in r-IFN-γ-treated early cultures, we selectively removed LC by a panning technique utilizing antibody OKT6. This panning technique has previously been shown to reduce the number of LC, which normally contribute 2–6% of the total population of epidermal cells, to less than 1% [12]. These cultures no longer contain LC as defined by monoclonal antibody staining and FACS analysis (Table I). Our data indicate that these cultured keratinocytes are synthesizing and expressing HLA-DR and not adsorbing shed antigens. However, we plan to measure direct biosynthesis of HLA-DR by these cells using [^{35}S]methionine labeled extracts and polyacrylamide gel electrophoresis.

Our results are consistent with previous reports that r-IFN-γ induced expression of HLA-DR in human melanoma cell lines [9] and cultured human vascular endothelial cells [10]. The concentrations of r-IFN-γ as well as the temporal appear-

TABLE I. *Effect of recombinant gamma interferon on cultured human keratinocyte HLA-DR expression*

A. Keratinocyte cultures[a]	*HLA-DR staining*	
Experiment	Mean[b]	% Stained[c]
Experiment 1		
Control	0.5	0
Gamma interferon treated[d]	130.0	72.0
Experiment 2		
Control	0	0
Gamma interferon treated	68.0	64.0
Experiment 3		
Control	0	0
Gamma interferon treated	84.0	54.0
B. LC-depleted keratinocyte cultures[a]	*HLA-DR and OKT-6 staining*	
Experiment	Mean[b]	% Stained[c]
HLA-DR		
Control	1.0	1.0
Gamma interferon treated	89.0	50.0
OKT6		
Control	0	0
Gamma interferon treated	5.0	0

[a] Nonspecific and Fc receptor-mediated background staining has been subtracted.

[b,c] Mean expression of fluorescence per cell and percent of cells with detectable staining were analyzed by FACS on setting: fluorescence = 2, scatter = 1, photomultiplier tube 650.

[d] 100 units/ml.

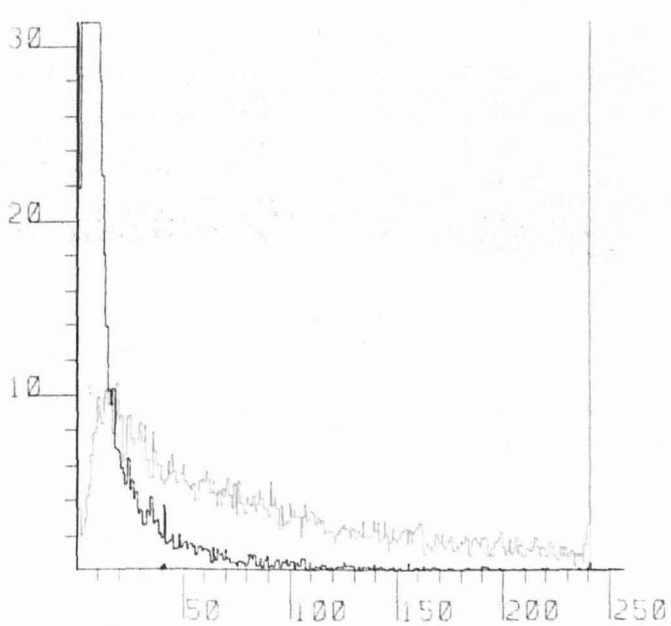

FIG 1. Histogram of r-IFN-γ-treated cultured keratinocytes labeled with anti-HLA-DR antibody plus goat antimouse-FITC (*top curve*) or a control immunoglobulin of IgG_{2a} isotype plus goat antimouse-FITC (*bottom curve*). The *vertical axis* shows fraction of total cells (10^{-3}) per channel; the *horizontal axis* shows fluorescence intensity in linear units.

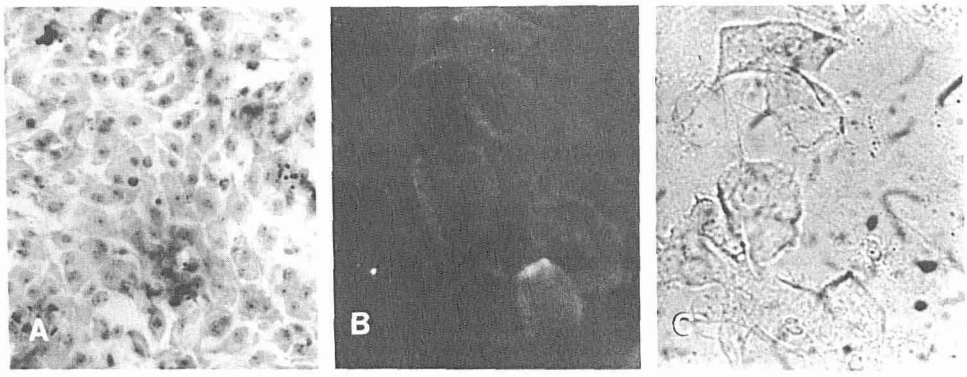

FIG 2. *a*, LC-depleted keratinocyte cultured with r-IFN-γ, (100 U/ml) fixed and stained with Giemsa on day 3 (× 328). *b*, LC-depleted keratinocyte cultured with r-IFN-γ (100 U/ml) stained in situ showing HLA-DR staining pattern on day 4 (× 754). *c*, Identical phase microscopic field (× 754).

ance of HLA-DR on the cultured cells are similar. However, by contrast to the other two cell types, in the keratinocyte cultures treated with r-IFN-γ an HLA-DR-negative subpopulation (10–30%) was always seen. This could be due to very low staining intensity which cannot be distinguished over background controls using the FACS or may reflect a true subpopulation of cells which, for example, are lacking the receptor for r-IFN-γ. Considering that keratinocyte cultures consist of cells in various stages of differentiation, the latter explanation may be more plausible.

Activated T lymphocytes produce γ-interferon, and therefore, it is possible that these cells mediate HLA-DR expression by keratinocytes in vivo [7]. Although our current work indicates that r-IFN-γ does not affect HLA-DR expression in LC, it is conceivable that, under certain disease conditions, LC shed HLA-DR in vivo and thereby coat neighboring keratinocytes with this antigen (T Basham, W Smith, L Lanier, V Morhenn, and T Merigan, submitted for publication). Whatever the exact mechanisms, it seems likely that γ-interferon plays an important role in regulating keratinocyte HLA-DR expression in a variety of skin diseases where expression of HLA-DR may be necessary for both antigen presentation and induction of cytolytic T lymphocyte responses.

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